On the Structural Differentiation of the Protozoa as seen in Microscopic Sections.

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(Plate XXVII.)

Our knowledge of the more minute structure of the Protozoa has considerably increased of late, this increase being no doubt due, in a great measure, to the introduction of the new apochromatic lenses as well as to the modern methods of fixation and differential staining. It is by these means that the protoplasmic contents of *Amœba*, for example, have been resolved into something more than a structureless hyaloplasm and its suspended granules; and we have grown conscious of an immensely complicated vacuolation stretching from the large ingestion-spaces on the one hand to the ultimate "Schaumplasm" [the heterogeneous foam structure of Bütschli] on the other. And, again, it is in the internal tensional activities of such structures that this author seeks for a possible mechanical substratum of some of the simpler vital phenomena. More recently, and apart from all hypotheses, F. Schütt *, by means of optical sections through a number of *Peridinia*, has not only demonstrated the existence of remarkable spaces ("Saftkammern"), but a veritable excretory system in connexion with them; while Prof. Greeff †, by substituting actual sections of *Amœbæ* for the optical sections of *Peridinia*, has brought to light a radial structure extending through the ectosarc and intimately connected with the contractility of the animal as a whole. In fact, if his observations can be confirmed, they mark the starting-point of a new era in our knowledge of the minute Protozoan anatomy.

It is with an extension of such an analysis, by sections of the ultimate Protozoan structure, that the following short contribution is concerned. Undoubtedly the material at my disposal will appear very limited; but the character of the technique became so forbidding in the case of forms not attainable in vast numbers,

* 'Sitzungsberichte der königlich preussischen Akademie der Wissenschaften zu Berlin,' 1892, xxiv. pp. 377–383.

† Dr. Greeff, "Ueber den Organismus der Amöben," Sitzungsberichte der Gesellschaft zur Beförderung der gesammten Naturwissenschaften zu Marburg, 1890, pp. 21–25.

that anything like even a preliminary survey of the leading Infusorian types would occupy much time, even years: however, as the results with some of those more easily manipulated were novel and interesting in themselves, I thought it worth while to publish them as a short preliminary essay.

After some trials I succeeded in obtaining sections in series of *Spirostomum*. The animals were killed by osmic acid, or by heating and afterwards fixing in Flemming's or Hermann's solution, in which they remained for twelve to eighteen hours, and were then transferred to a tall tube, from which the supernatant liquid was repeatedly decanted and replaced by distilled water for some hours. After the last filling up the water was poured off and alcohol added very gradually, in order to prevent contraction of the delicate sarcode, until a 50-per-cent. solution was reached, in which the Infusoria remained like a coarse precipitate at the bottom for twelve to eighteen hours more. The strength of the spirit was then increased until the whole was gradually replaced by absolute alcohol. It made no difference as to the ultimate result whether the objects were treated with cedar oil or chloroform and then transferred to paraffin. So soon as the Infusoria had settled in this last and it had been replenished two or three times, the larger part of the fluid was poured off and the remainder, with the Infusoria, shaken up slightly and cast in the usual manner. The sections were cut in the usual way with a Minot microtome and stained as required.

Spirostoma treated in the above manner and stained with either gentian-violet, Victoria blue, or orange, showed a splendid reticulum or vacuolation (quite invisible except in section) of the whole protoplasmic body (sarcode) (Pl. XXVII. figs. 2, 3, 4). The meshes of this reticulum were finer round the nuclei and largest between these structures and the periphery, where they died down to a compact protoplasmic layer just within the actual outer membrane. This layer undoubtedly corresponds to what Schütt described in *Peridinium* as the "Hautschicht." It is no less obvious that the large vacuolations correspond to his "Saftkammern" and the intervening reticulum to his "Füllplasma." The remarkable radial structures of Greeff were not apparent, but something, at least, suggestive of his "glänzende Punkte" [fine refracting particles], in which this radial structure terminated, are seen underlying the apices of the longitudinal ridges (fig. 8, *a*).

These sections appear to indicate the existence of denser tracts

of substance running down the entire length of each ridge, or in reality an apparently resistant structure which may have much to do with the rapid undulations and contractions of the animal when in activity. Still more interesting in relation to these appearances were a series of sections across the oral furrow from its anterior extremity until it finally dips into the inner sarcode by a distinct ostium (fig. 4). This furrow contains at its bottom a little ridge; and immediately below this is a dense rod similar to those above, only much larger in section (fig. 6, *a*). The ridge bears, above, the well-known oral cilia along its whole length, and the rod appears to act as a support for the whole ciliary apparatus. The sarcode is more firmly in connection with the inner edge of this rod than the surrounding parts, and there appear faint refractive lines running from this inwards (fig. 6, *b*). I cannot understand the significance of these refractive striæ unless the fibrillation represents the agent in transmission of something like a nervous impulse from the inner protoplasm to a specially mobile region.

When subjected to a precisely similar treatment, the protoplasmic body of *Paramœcium* shows no such differentiation into a reticulum of chromatic fibres and achromatic spaces as that described above. Their contents are far more nearly uniformly made up of granular protoplasm imbedded in which are seen the food-stuffs as more deeply-staining masses. At the same time there do appear about the huge macronuclei irregular spaces and lacunæ having no immediate connexion with the contractile vacuole, which are undoubtedly analogous to the more numerous spaces ("Saftkammern") in *Peridinium*. Consequently they must be looked upon as all that is left of the splendid protoplasmic network of the *Spirostoma*.

The dying out of the necessity of such a reticulum, through forms like *Peridinium* to the *Paramœcia*, is a very curious fact, and it shows, I think, that we have not yet done with protoplasmic reticula, at any rate in the sense of their significance.

Irregularly disposed throughout all the internal reticula of *Spirostomum* are innumerable small refractive dots. They appear along the fibres of the network and become more numerous towards the periphery on all sides (fig. 3). With care they are to be seen in the interior of the living animal, and in sections appear closely connected with the double spiral rows of dots characteristic of the species. I have elsewhere* described the

* Ann. & Mag. N. Hist. 6th ser. vol. xi. p. 149.

relation existing between the primarily ingested food in *Amœbæ* and the so-called crystalline bodies underlying the ectosarc, and have suggested that they may be the insoluble residue of the animal's prey. Some observations (as yet unpublished) of my friend Mr. Bernard on the digestive cells of the blood-sucking Arachnids have strongly confirmed this view, and it seems probable that these refractive dots are the last residuum of the animal's food, and that as such they make their way out along the lines of the enclosing membrane.

I have hitherto made no allusion to any relation existing between the condensations I have described beneath the external rugæ and Haeckel's myophan striation, simply because the more closely I sought after it the more distant any such relationship appeared: in fact these structures appear to be rather complementary or antagonistic than homologous. As Haeckel describes them, the myophan striations "erscheinen als ein System von regelmässigen, parallelen, finen Streifen ..... dicht gedrängt neben einander verlaufen, und abwechselnd heller und dunkler erscheinen" *; and according to this author, these structures lie at the base of his ciliary or second layer, and themselves constitute the third layer †. Now the condensations I have described are embedded in the ciliary layer itself, and are immediately below the not very distinct outer cuticle. In somewhat tangential sections the myophan striæ are seen to correspond exactly with the ditches between the superficial ridges, a fact pointed out by Entz ‡; while the denser rods described above are situated immediately beneath the apices of these ridges, and they consequently alternate with the myophan stripes. Thin sections do not demonstrate the existence of such contractile fibres forming a third layer, and the homogeneous character of the condensations to which I have drawn attention seem to suggest elastic, in contradistinction to contractile function, and to shift the responsibility of the latter on to the ciliary layer as a whole. It may here be remarked that this view would bring the contractility of the ciliate Infusoria into much closer harmony with that of the Rhizopods.

Since describing the cross section of the oral furrow and the comparatively large skeletal structure which lies beneath it, I

* 'Jenaische Zeitschrift für Medecin,' Bd. vii. p. 535.

† Greeff considers them as hollow structures and speaks of the "Lumina der Muskelfasern."

‡ 'Zeitschrift für wissenschaft. Zoologie,' xxxviii. p. 167.

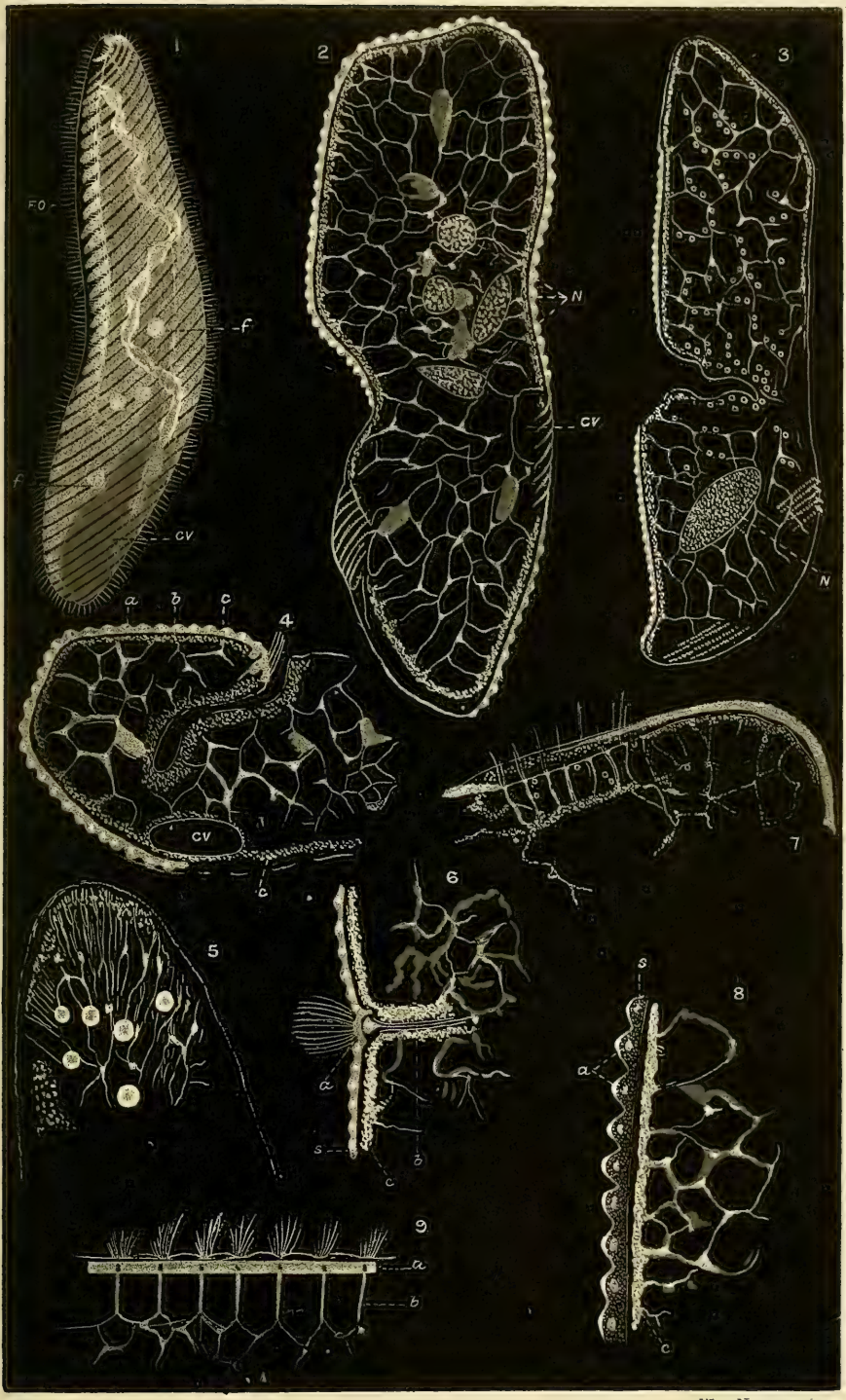
have made longitudinal sections of the same region. In these there appear, stretching across the denser band, refractive stripes which terminate externally in successive fans of cilia seen edgewise, while internally these ciliary roots, so to speak, are related to a remarkable series of protoplasmic bodies terminating interiorly in the general network (fig. 7).

I have diagrammatically represented the relations of all these structures (fig. 9). It will be seen that the rods *b* (fig. 7) are the longitudinal expression of the fine striæ seen passing inwards from the fan of cilia (fig. 6, *b*), and stand as a means of connexion between the inner network and the bases of the cilia themselves. Whatever their function, I have now observed them in the living animal, and they appear to be permanently related to the ciliary apparatus.

EXPLANATION OF PLATE XXVII.

Fig. 1. *Spirostomum*, showing the relation of the oral furrow, *FO*.
2. Section showing protoplasmic network.
3. The same, showing refractive bodies between the spaces.
4. Section through mouth.
5. Section with food-matter recently ingested.
6. Cross section of oral furrow.
7. Longitudinal section of oral furrow.
8. Section showing the relation of the outer layers.
9. Diagram of the oral cilia and their related parts.

a. Dense rods in ciliary layer.
b. Protoplasmic bodies beneath the ciliary apparatus of pharynx.
c. Dense outer layer of inner protoplasm.
c.v. Contractile vacuole.
n. Nucleus.
f. Food-masses.



M.P. Parker lith. West, Newman imp.

STRUCTURAL DIFFERENTIATION OF PROTOZOA.